

**(19) Japanese Patent (JP)**

(11) Publication number: 63-196241

**(12) Publication of Patent Application**

(43) Date of publication of application: 15.08.1988

(51) Int. Cl.: A23L 1/23 JPO file number: 6946-4B

A23C 19/086 JPO File number: 8114-4B

(54) Title: Process for the production of a Cheese Flavour

(21) Application number: 63-11020

(22) Filing date: 22.01.1988

(32) Priority data: 23.01.1987

(33) West Germany (DE)

(31) Application file: P 37 01 836.1

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Patent description

1. Title: Process for the production of a cheese flavour
2. Scope of claims
  1. Method for the production of a cheese flavour characterized in that the cheese flavour is derived from a dairy product by incubating a dairy product in a thin layer with cheese-flavour -producing microorganisms.
  2. A method in accordance with claim 1, characterized in that the dairy product used is cheese curd, yoghurt, cottage cheese or powdered milk in the form of a paste.
  3. A method in accordance with claim 1 or 2, characterized in that the dairy product is absorbed through a porous support material.
  4. A method in accordance with claim 3, characterized in that the porous support material is made of either clay or plastic.
  5. A method in accordance with claims 1 to 4, characterized in that the cheese-flavour-producing microorganisms are yeasts of the genera *Torulopsis* and/or *Candida* or mould of the genera *Penicillium*.
  6. A method in accordance with claim 5, characterized in that the incubation step uses *Torulopsis etchellsii* and/or *Candida lipolytica* and *Penicillium roqueforti* or *Penicillium camemberti*.
  7. Either one of the methods in accordance with claims 1 to 6, characterized in that Lipase or a Lipase producing microorganism is added at the time of incubation.
  8. Either one of the methods in accordance with claims 1 to 7, characterized in that the incubation temperature ranges between 10 and 30° C.
  9. A method in accordance with claim 8, characterized in that the incubation temperature is between 15 and 25° C.
  10. A cheese flavour obtained using either one of the methods in accordance with claims 1 to 9.
  11. The use of a cheese flavour in accordance to claim 10 in salad sauces, mayonnaises, snacks and baked goods.

### **3. Detailed description of the invention**

The importance of the use of natural flavours has been increasing. However, typically, the maturing process used to produce a blue cheese flavour takes several months and therefore requires an extensive amount of time. For this reason, various attempts have been made to accelerate fermentation in the flavour production process. Prior art includes methods using fat fractionation, Lipase or mould fungi contained in milk cultures with Lipase producing microorganisms especially by submerged fermentation of *P. roqueforti*. The flavour resides in the layer of fat or is obtained by drying the fermented mixture. However, until now it has not been possible to achieve a quality comparable to that of original blue cheese using these methods. Other publications detailing methods used in this art include:

GB 10 57 170; GB 13 61 817; DE 15 17 133; DD 14.74.99; US 31 00 153; US 36 67 968; US 39 73 042; US 41 33 895; US 37 20 520; B. K. Dwivedi and J. E. Kinsa, *J. of Food Science* (1974), p. 620; R. Jolly and F. V. Kosikowski, *J. of Food Science* (1975), p. 285; J. N. Nelson, *J. Agr. Food Chem.* (1970), p. 567; H. Ruttloff, A. Quehl, A. Leuchterberger, *Die Nahrung* (1984), p. 873.

In the above-mentioned patents and patent applications the Roquefort flavour is typically associated with compositions that are high in ketones. The precursor enzymes and the microorganisms that are added are typically selected solely based on the increase in ketone content. However, these methods do not consider the fact that the original Roquefort cheese flavour is composed of a variety of components.

The European Patent publication number 01 84 105 mentions, for example, the preparation of blue cheese flavour giving substances that can be added to salad sauces, mayonnaises, snack and baked goods. This publication explains a method wherein yeasts of the genera *Torulopsis* were inoculated to milk first. Once the curd had formed the whey was removed and the curd was inoculated with *Penicillium Roqueforti*. It is also possible to simultaneously inoculate the milk with both microorganisms. The cheese flavour can be harvested 3 to 4 days later. However, the flavour obtained will have a concentration 4 to 5 times higher than in conventionally matured Roquefort.

It also became clear that it is possible to obtain a wide spectrum of flavour and greatly increase the cheese flavour yield by incubating dairy products in a thin layer with cheese flavour producing microorganisms.

Thus, the present invention relates to a method for the production of a cheese flavour wherein a cheese flavour is obtained from a dairy product by incubating dairy products in a thin layer and fermenting it with cheese flavour producing microorganisms as well as to cheese flavours produced using this method.

Preferred embodiments as well as the scope of claims of the present invention will become apparent from the detailed description given hereinafter.

All suitable dairy products, known to people skilled in the art, can be used as starting material. However, the use of curd, yoghurt or cottage cheese as well as milk powder in the form of paste is preferred. These products have the advantage that contrary to conventional cheese production they no longer need to go through the curd-forming step. The starting material is spread into a thin layer or is preferably absorbed through a porous support material. Suitable support materials include, for example, porous support materials made of plastic or clay, especially low expansion clay such as stones used for hydroponic cultivation. Since the processing efficiency is linked to the size of the surface area, advantageously, the support material is shaped so that the dairy product can be efficiently absorbed on a large surface. Preferably the support material is spherical.

The starting culture media is thinly spread on the support material to make surface fermentation possible. This is achieved by, for example, mixing a low expansion clay aggregate with the dairy product, and blending both components using a drum mixer, and then eliminating the lumps from the excess dairy product.

The dairy product is then incubated with cheese-flavour producing microorganisms that produce preferably a mould fungus. Each of the yeasts of the genera *Torulopsis* and/or *Candida* as well as flavour-producing mould fungus of the genera *Penicillium* can be used. The dairy product can be inoculated at the time when the starting material is applied on the support material before or after as is suitable. Besides, it is also possible to inoculate the starting material first, then add the lipase, incubate the mixture for a few hours, absorb it through the support material and then inoculate the dairy product with *Penicillium*.

Yeasts that are particularly effective are yeasts of the genera *Torulopsis* and *Candida* particularly *Torulopsis etchellsii* and *Candida lipolytica* as well as yeasts with metabolic properties comparable to those of *Torulopsis* and *Candida* such as *T. holmii*, *T. humilis*,

*T. torresii*, *T. vanderwaltii*, *T. versatilis* and *T. wickerhamii* and/or *Candida cylindracea* and *C. rugosa*.

Preferred used mould fungus of the genera *penicillium* includes *Penicillium roqueforti* as well as *Penicillium camemberti*. These funguses can be effortlessly isolated from a suitable kind of cheese by an expert.

The amount of yeast or *Penicillium* inoculum to be used is dependent on the activity of the bacterial strain, which is determined through simple preliminary tests. The amount of inoculum may be changed within certain limits and the process accelerated by using higher concentrations of the inoculum.

If necessary, lipase may be added to the fermentation culture in the amounts of 0.01 to 1.0%, preferably 0.1 to 0.2%. Lipase-producing microorganisms of the genera *Penicillium*, *Aspergillus*, *Mucor*, *Rhizopus* or *Candida* may be used instead of Lipase.

The fermentate is fermented between +10° C and 30° C preferably between 15° C and 25° C.

The quality of the cheese flavour obtained 5 to 7 days later varies according to the type of *Penicillium* fungus used to cultivate the culture media. For example, selecting *Penicillium roqueforti* derived from Roquefort cheese will result in a flavour with a strong "blue cheese" taste. However when using a *Penicillium roquefortii* derived from a milder blue cheese such as Danablu or Rambol, the flavour obtained is described by specialists as an ordinary cheese flavour. Using *Penicillium camemberti* results in an overall Camembert taste.

In order to condense the flavour derived from the fermentate, the culture media is extracted from the support material using a lower alcohol, preferably methanol, and/or water. The fermentate is then homogenized and the solid contents diluted in water. Next, the mixture is extracted using a non-polar solvent such as and preferably pentane or methylene chloride. The flavour may also be directly extracted using a non-polar solvent, for example pentane or a mixture of pentane and methylene chloride.

Hereafter, the present invention will be explained by referring to Examples. The amounts of inoculum are commercially available or have been obtained from experiments that used readily available strains. Unless otherwise indicated the percentages are by weight.

### Example 1

The pre-incubation solutions of *Torulopsis etchellsii* and *Penicillium roqueforti* were prepared using the following culture media and incubated for 2 and then 3 to 5 days, at 25 °C.

40 g/l malt extract

4 g/l yeast extract

20 g/l glucose

1 g/l (NH4)2HPO4

20 kg of curd was mixed with 1 l culture of *Torulopsis etchellsii* and incubated for 48 hours at 25° C. For the purpose of surface fermentation, the mixture was then transferred to a flask allowing better temperature control. The culture was subsequently inoculated with 1 l of the *Penicillium roqueforti* culture solution derived from Roquefort. Next, the culture was matured for 5 days at 25° C.

The culture was then mixed with 20 l of methanol and homogenized. 200 l water was subsequently added and any solid content eliminated. The solution was then extracted using 25 l of pentane/ methylene chloride (2: 1, v: v). The solvent was then eliminated through distillation.

The blue cheese flavour obtained was 10 to 15 times stronger than that derived from Roquefort cheese using the same method.

### Example 2

Using the same method as in example 1, the *Penicillium roqueforti* derived from Roquefort was replaced with *Penicillium roqueforti* derived from Rambol cheese; the flavour obtained was that of an "ordinary cheese". When using *Penicillium camemberti* derived from Camembert cheese the flavour had an overall Camembert taste. In each case the concentrations were the same as the ones used in example 1.

Example 3

The mixture was prepared using the same method as the one described in example 1 except where it was mixed with a low expansion clay aggregate (Stones used for hydroponic cultivation), and blended with the other components using a drum mixer. After eliminating the lumps from the excess curd cheese the mixture was incubated using *Penicillium roqueforti* as described in example 1.

The blue cheese flavour obtained was 15 to 20 times stronger than that derived from Roquefort cheese using the same method.

Example 4

The mixture was prepared using the same method as the one described in example 3 except where *Torulopsis etchellsii* was replaced by *Candida lipolytica*. The blue cheese flavour obtained was 10 to 12 times stronger than that derived from Roquefort cheese.

Example 5

The mixture was prepared using the same method as the one described in example 1 except where 0.1% lipase derived from *Candida cylindracea* (Sigma Chemical Co.) was added to the curd cheese. The blue cheese flavour obtained was 15 to 20 times stronger than that derived from Roquefort cheese.

Translators' note: the last page states "Continuation from Page 1. We have therefore included this on page 1